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Research Article

Dietary L-Carnitine Alleviates the Adverse Effects Caused by Reducing Protein and Increasing Fat Contents in Diet Juvenile Largemouth Bass (*Micropterus salmoides*)

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Protein ingredients for formulation of fish feeds are expensive and have limited availability. Therefore, reducing dietary protein while increasing dietary fat content is a common practice in rearing carnivorous fish species. However, the ability of dietary L-carnitine to alleviate adverse effects in such diets is currently unknown. This study investigated the role of L-carnitine supplementation in alleviating adverse effects on growth performance, energy metabolism, antioxidant capacity, and inflammation response in juvenile largemouth bass (*Micropterus salmoides*) fed on a low protein and high fat diet. Three diets were formulated to contain low protein and high fat (LPHF: 420 g kg^{-1} protein and 150 g kg^{-1} lipid), LPHF supplemented with L-carnitine (LPHFC: 420 g kg^{-1} protein and 150 g kg^{-1} lipid), and a control diet (CON: 480 g kg^{-1} protein and 130 g kg^{-1} lipid). The diets were fed to 30 largemouth bass (10.75 ± 0.01 g) juveniles in triplicates for eight weeks. The results showed that the fish feed on LPHF diet increased hepatosomatic index, visceral somatic index, mesenteric fat index, whole-body crude fat content, serum and liver triglyceride concentrations, and serum non-esterified fatty acid level than those fed on CON diet. Moreover, the fish fed on LPHF diet increased serum alanine aminotransferase activity and liver malondialdehyde content and reduced superoxide dismutase (SOD) activities in the serum and liver. Furthermore, the fish fed on LPHF diet reduced the whole-body crude protein content. Interestingly, feeding the fish on the LPHFC diet decreased fat deposition and liver damage by downregulating the expression of genes related to lipogenesis, inflammation, and increased SOD activity. This study indicates that L-carnitine supplementation in largemouth bass alleviates the adverse effects caused by LPHF diet by decreasing lipogenesis and increasing lipid catabolism. Our study provides novel knowledge on strategies to improve utilization of LPHF diet in cultured aquatic animals.

1. Introduction

Currently, protein is the most expensive nutrient in fish feed formulation [1], and its sources are limited [2]. Dietary fat is an important nutrient, providing essential fatty acids and energy for growth in cultured fish [3]. Accordingly, high-fat diets (HFD) are widely used in aquaculture to maximize "protein-sparing effect" [4, 5]. Accordingly, increasing dietary fat content within a certain range can promote growth and protein deposition in some fish species such as blunt snout bream (*Megalobrama amblycephala*) [6] and Nile tilapia (*Oreochromis niloticus*) [7]. However, the use of HFD also causes many negative effects, such as impaired lipid metabolism, leading to excess fat deposition in the whole

body and organs, such as the liver and adipose tissue in cultured fish [8, 9]. Moreover, HFD damaged immunity and antioxidant systems in juvenile black seabream (*Acanthopagrus schlegelii*) [10] and juvenile largemouth bass (*Micropterus salmoides*) [11]. Therefore, studying approaches to alleviate the adverse effects caused by feeding fish with HFD is an important topic in aquaculture nutrition.

L-carnitine (L-3-hydroxy-4-N–trimethylaminobutyrate) is an essential metabolite in eukaryotic cells for lipid metabolism. L-carnitine transports long-chain fatty acids (LCFAs) from cytosol into mitochondrial matrix for subsequent β-oxidation and modulation of the intracellular acyl-CoA/CoA ratio [12, 13]. In animals, L-carnitine can be synthesized from lysine and methionine in the liver [14, 15]. Nevertheless, dietary exogenous L-carnitine supplementation is still necessary because endogenous synthesis is inadequate for lipid metabolism, especially in fish fed on HFD [16–18]. Previous studies in mammals and some fish species have confirmed that dietary L-carnitine effectively promote growth and lipid catabolism, reduce hepatic lipid deposition, and ameliorate body damage [10, 19]. Evidently, L-carnitine supplementation reduced the whole-body lipid content from 5.95% to 4.89% and significantly promoted growth in Asian catfish (*Clarias batrachus*) fry [20]. Similarly, dietary L-carnitine addition also elevated significantly mitochondrial β-oxidation activity, reduced fat accumulation, and increased protein deposition in Nile tilapia [21] and zebrafish (*Danio rerio*) [19, 22]. On the contrary, inhibition of L-carnitine endogenous synthesis in Nile tilapia or knockout of carnitine palmitoyl-CoA transferase I (CPT-I), which is the key enzyme for transporting carnitine-acyl-CoA into mitochondria in zebrafish, suppressed mitochondrial β-oxidation, increased fat deposition in the body, especially in the liver, and also impaired immunity response [22, 23]. The results from the above studies suggest that, L-carnitine supplementation might be a promising strategy to reduce excess fat deposition in cultured aquatic species.

However, most previous studies on L-carnitine supplementation in fish used diets with optimum nutrients levels. The role of L-carnitine supplementation in fish fed on diets containing low protein and high lipid contents is currently not known. Moreover, a few studies also reported that the lipid-lowering effects of L-carnitine were not obvious in some fish species [24, 25]. Therefore, more studies are required to evaluate the effects of dietary L-carnitine supplementation considering that distinct fish species have different physiological metabolism.

The largemouth bass (*Micropterus salmoides*) is a carnivorous valuable aquatic species extensively cultured in China (China Fishery Statistical Yearbook, 2018). The species require high protein diet (480 to 520 g kg^{-1}) with lipid level ranging from 80 to 130 g kg^{-1} due to its carnivorous feeding habit [26, 27]. However, dietary protein sources for largemouth bass required for feed formulation is also affected by the dwindling wild fisheries resources. Therefore, reducing dietary protein while increasing dietary fat content has been a common trend in commercial largemouth bass feed formulation. However, high dietary fat content also causes excess fat accumulation in the liver of largemouth bass [28, 29]. A previous study showed that dietary L-carnitine supplementation improved growth performance, lipid metabolism, and liver health in juvenile largemouth bass in which dietary fish oil was replacement by soybean oil [30]. However, the ability of dietary L-carnitine to alleviate adverse effects in a diet with lower protein and higher fat contents in largemouth bass is currently not well understood. Moreover, whether the ability of L-carnitine to alleviate the adverse effects in such a diet could strengthen the "protein sparing effect" is also unknown.

Therefore, the present study investigated the ability of L-carnitine to alleviate the possible adverse effects caused by reducing dietary protein and increasing lipid contents in the liver of largemouth bass. To achieve our objective, we used practical feed ingredients to formulate a control diet (520 g kg^{-1} protein and 130 g kg^{-1} fat), a "low protein and high fat diet" (450 protein g kg^{-1} and 150 g kg^{-1} fat, LPHF), and the LPHF diet supplemented with L-carnitine (LPHFC). The three diets were fed to juvenile largemouth bass for eight weeks. The ability of L-carnitine to alleviate the adverse effects were assessed by determining growth performance and assaying lipid, glucose and protein metabolism, oxidative stress, and liver health indexes. This study provides useful information in feed formulation for improving utilization of LPHF in cultured carnivorous species such largemouth bass.

2. Materials and Methods

2.1. Animal Ethics Statement. All experiments were conducted under the Guidance of the Care and Use of Laboratory Animals in China. This research was approved by the Committee on the Ethics of Animal Experiments of East China Normal University (Approval ID: f20201002).

2.2. Experimental Fish, Diets and Study Design. Juvenile largemouth bass were purchased from Guangzhou Yufeng Fry Breeding Base (Guangzhou, Guangdong, China). Before the formal experiment, all fish were acclimated into two 7500 L tanks under a natural photoperiod at 27.00 ± 1.00°C for four weeks. During this time, the fish were fed by using a commercial diet (Tongwei, Chengdu, Sichuan province, China) containing ≥470 g kg^{-1} protein and ≥140 g kg^{-1} lipid. Three diets with similar gross energy content were formulated by using practical feed ingredients commonly used in feeding cultured species in China (Table 1). Dietary gross energy was determined by using the energy constants of digestive carbohydrate (4.1 kcal g^{-1}), protein (5.4 kcal g^{-1}), and lipids (9.5 kcal g^{-1}) [31]. The ingredients were used to formulate a control (optimum nutrients) diet containing approximately 480 g kg^{-1} protein and 130 g kg^{-1} lipid, a low protein and high fat (LPHF) diet containing approximately 420 g kg^{-1} protein and 150 g kg^{-1} lipid, and a LPHF diet supplemented with 500 mg kg^{-1} L-carnitine (LPHFC). The amount of L-carnitine was based on previous studies in Nile tilapia [32] and Amur minnow (*Phoxinus lagowskii*) [33]. The diets were made and stored following a previous study in our laboratory [34]. Three samples of each diet were used for proximate composition analysis as described in the next section.

Table 1: Formulation and proximate composition of experimental diets (g kg^{-1} dry matter).

Ingredient	Experimental diets		
	CON	LPHF	LPHFC
Fishmeal	450	400	400
Chicken meal	100	80	80
Soybean meal	130	120	120
Wheat flour	110	110	110
Wheat gluten	20	20	20
Cassava starch	30	50	50
Soybean oil	60	90	90
Squid paste	40	40	40
$Ca(H_2PO_4)_2$	15	15	15
Vitamin premix[a]	5	5	5
Mineral premix[b]	10	10	10
Cellulose	30	60	59.5
L-carnitine			0.5
Total	**1000**	**1000**	**1000**
Proximate composition			
Dry matter	919.3	914.9	914.9
Crude protein	476.92	420.07	427.26
Crude lipid	132.10	151.20	157.40
Ash	99.87	96.22	102.58
Gross energy (kcal g^{-1})[c]	5.24	5.30	5.40

[a]Vitamin premix (mg or IU/kg): 500,000 I.U. (international units) vitamin A, 50,000 I.U. vitamin D3, 2500 mg vitamin E, 1000 mg vitamin K3, 5000 mg vitamin B1, 5000 mg vitamin B2, 5000 mg vitamin B6, 5000 μg vitamin B12, 25,000 mg inositol, 10,000 mg pantothenic acid, 100,000 mg cholin, 25,000 mg niacin, 1000 mg folic acid, 250 mg biotin, and 10,000 mg vitamin C. [b]Mineral premix (g/kg): 314.0 g CaCO3; 469.3 g KH2PO4; 147.4 g MgSO4·7H2O; 49.8 g NaCl; 10.9 g Fe (II) gluconate; 3.12 g MnSO4·H2O; 4.67 g ZnSO4·7H2O; 0.62 g CuSO4·5H2O; 0.16 g KJ; 0.08 g CoCl2·6H2O; 0.06 g NH4 molybdate; and 0.02 g NaSeO3. [c]Dietary gross energy was determined by using the energy constants of digestive carbohydrate (4.1 kcal g^{-1}), protein (5.4 kcal g^{-1}), and lipids (9.5 kcal g^{-1}).

After acclimation, 270 visually healthy largemouth bass with relatively similar initial mean weight (10.75 ± 0.01 g) were selected and randomly distributed into nine 200 L tanks (three replicates per treatment, 30 fish per tank) in an indoor recirculating freshwater aquaculture system. Each three tanks were randomly allocated with fish and fed with the experimental diets at 5% of their body weight per day in the morning (8 : 30 hours) and evening (17 : 30 hours) every day for eight weeks. The amount of feed fed to the fish on daily basis was recorded for calculation of feed utilization. During the whole study period, water temperature was maintained at 27.00 ± 1.00°C, dissolved oxygen was not less than 6.0 mg/L, and total ammonia was less than 0.05 mg/L under natural light cycle (12 h/12 h light/dark cycle).

2.3. Evaluation of Fish Growth Performance and Feed Utilization. At the end of the experiment, all fish were fasted overnight. The fish in each tank were counted and bulk weighed to calculate survival rate (SR) and weight gain (WG). The body weights and lengths of 18 fish from each treatment (six fish per tank) were individually measured. The SR, WG, feed conversion ratio (FCR), condition factor (CF), and protein efficiency ratio (PER) were calculated by using the following formulae:

$$\begin{aligned}
\text{SR}\,(\%) &= 100 \times (\text{Final number of fish/Initial number of fish})\\
\text{WG}\,(\%) &= 100 \times (\text{Final mean body weight} - \text{Initial mean body weight})/\text{Initial mean body weight}\\
\text{FCR} &= \text{Total amount of feed fed/Total wet weight gained}\\
\text{CF}\,(\%) &= 100 \times \text{Final fish weight}/(\text{Final fish length})^3\\
\text{PER} &= \text{Weight gain/Total amount of protein fed}
\end{aligned} \tag{1}$$

2.4. Fish Body Composition Analysis. A sample of six fish from each tank (18 fish per treatment) was randomly collected and kept at -20°C for body composition analysis. The fish proximate body composition and the experimental diets were analyzed according to the standard method [35]. The moisture was determined by drying the samples in an oven at 105°C until reached a constant weight. Ash content was determined by incinerating the samples in a muffle furnace at 550°C for 12 hours. Crude protein was determined by measuring nitrogen by using the Kjeldahl method in FOSS Kjeltec 8200 (Kjeltec, Foss, Sweden). Crude lipid was determined by Soxhlet extraction method [36].

2.5. Blood Collection and Determination of Organ Indexes. A sample of 18 fish from each treatment (six fish per tank) was randomly collected, anesthetized by using MS222 (20 mg/L), and sacrificed for tissue sample collection. Before tissue collection, blood was withdrawn from the caudal vein by using 2 mL syringes (Klmedical, China) and centrifuged (1500 rpm, 10 min). The resulting serum was immediately frozen at −80°C for biochemical analysis as indicated in the next section. After blood collection, individual fish were weighed and dissected to obtain viscera, liver, and mesenteric fat. The viscera, liver, and mesenteric fat were weighed to determine viscera somatic index (VSI), hepatosomatic index (HSI), and mesenteric fat index (MFI) by using the following formulae:

$$\begin{aligned}
\text{VSI}\,(\%) &= 100 \times (\text{Viscera somatic weight/Body weight})\\
\text{HSI}\,(\%) &= 100 \times (\text{Liver weight/Body weight})\\
\text{MFI}\,(\%) &= 100 \times (\text{Mesenteric fat weight/Body weight})
\end{aligned} \tag{2}$$

2.6. Biochemical Parameters Measurements. The liver and serum tissues from six fish per tank were immediately frozen in liquid nitrogen and stored at −80°C for biochemical analysis. The serum was used for triglyceride (TG) measurement, non-esterified free fatty acids (NEFA), total amino acids (T-AA), glucose, and lactate contents by using specific commercial assay kits (Nanjing Jiancheng Biotech Co., Nanjing, China) based on the manufacturer's instructions. Similarly, the activities of alanine aminotransferase (ALT), aspartate transaminase (AST), total superoxide dismutase (SOD), and total antioxidant capacity (T-AOC) content in the serum were also determined by using specific commercial assay kits (Nanjing Jiancheng Biotech Co., Nanjing, China). Finally, the TG, glycogen, malondialdehyde (MDA), and T-

AOC contents and SOD and catalase (CAT) activities in the liver were also assayed by using specific commercial assay kits (Nanjing Jiancheng Biotech Co., Nanjing, China). All these kits are suitable for fish sample assays and have been used widely in many fish studies [19, 22]. All results of these assays were recorded on a microplate reader (Epoch, BioTek, USA). The part of the liver was stored at −80 °C for extraction of total RNA.

2.7. Quantitative Real-Time PCR. The total RNA from six liver tissue per tank was extracted by using Trizol reagent (Beyotime, Shanghai, China) following the manufacturer's instructions. The quality and quantity of total RNA were estimated by using a Nano Drop 2000 Spectrophotometer (Thermo, USA). The cDNAs were synthesized by using a PrimerScriptTM RT reagent Kit (with gDNase) (Takara, Japan) following the manufacturer's instructions. The real-time quantitative PCR (qRT-PCR) was conducted by using CFX96 real-time RCR system (Bio-Rad, Richmond, CA) as described previously in our laboratory [34]. The β-actin was used as reference gene due to its stability. The primers used in the present study are shown in Table 2. The qRT-PCR results were estimated by the $2^{-\Delta\Delta Ct}$ method [37]. During analysis, the qRT-PCR efficiency was between 95% and 105% for all analyses. Each qRT-PCR run was performed in triplicate, and negative control (no cDNA) was also included.

2.8. Statistical Analyses. The data obtained were tested for normality by using Shapiro-Wilk test and homogeneity of variances using Levene's test. The significant differences on the measured parameters among the three diets for all data were evaluated by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for specific comparisons. The results with $p \leq 0.05$ were considered statistically significant. During reporting, the results are expressed as mean ± SEM (standard error of the mean). All data were analyzed by using SPSS Statistics 20.0 software (IBM, Armonk, NY, USA).

3. Results

3.1. Effects of Dietary L-Carnitine on Growth Performance and Organ Indexes. Growth performance of the juvenile largemouth bass fed on the three experimental diets are presented in Figure 1. Feeding the largemouth bass with the experimental diets did not affect significantly the SR (Figure 1(a)), WG (Figure 1(b)), and FCR (Figure 1(c)) ($p > 0.05$). However, the fish fed on LPHF and LPHFC diets increased significantly the PER compared to those fed on the CON diet (Figure 1(d)) ($p < 0.05$), with no significant difference in PER between them ($p > 0.05$). The fish fed on LPHF diet decreased the CF than those fed on CON diet (Figure 1(e); $p < 0.05$). However, feeding the largemouth bass on the LPHFC diet increased the CF than those fed on LPHF diet (Figure 1(e); $p < 0.05$) to a similar level as the CON diet ($p > 0.05$). Furthermore, the fish fed on the LPHF diet increased significantly the HSI (Figure 1(g)) and VSI (Figure 1(h)) compared to those fed on CON diet ($p < 0.05$). However, feeding the largemouth bass on the LPHFC diet decreased significantly HSI ($p < 0.05$) and VSI ($p < 0.05$) than those fed on LPHF to a similar level as in the CON diet ($p > 0.05$).

3.2. Effects of Dietary L-Carnitine on Lipid Accumulation. The largemouth bass fed on LPHF diet increased significantly the whole-body fat (Figure 2(a)), serum TG content (Figure 2(b)), serum NEFA content (Figure 2(c)), and TG in the liver (Figure 2(e)) than those fed on CON diet ($p < 0.05$). Interestingly, feeding the fish on the LPHFC diet decreased significantly the whole-body fat, serum TG content, and serum NEFA content than those fed on LPHF diet ($p > 0.05$) to a similar level to the CON diet ($p > 0.05$). Moreover, the fish fed on LPHFC diet decreased significantly the MFI than those fed on LPHF diet ($p < 0.05$), but higher than those fed on CON diet (Figure 2(d); $p < 0.05$). Notably, feeding the largemouth bass on the LPHFC diet decreased the TG content in the liver than those fed on LPHF diet ($p > 0.05$) to an even lower amount than the CON diet (Figure 2(e); $p < 0.05$). Obviously, the livers of fish fed on LPHF diet were white in color, and the livers of fish fed on CON and LPHFC diet were bright red in color (Figure 2(f)). The fish fed on LPHF diet increased the number and size of lipid droplets than those fed on CON diet (Figure 2(g)). However, dietary L-carnitine supplementation obviously reduced the number and size of lipid droplets in largemouth bass to a similar level as in the CON diet (Figure 2(g)).

3.3. Effects of Dietary L-Carnitine on mRNA Expression of Genes Related to Lipid Metabolism. Feeding the largemouth bass on the LPHF diet did not affect significantly most mRNA expression of genes related to lipogenesis [diacylglycerol O-acyltransferase 1 (DGAT1), fatty acid synthase 1 (FAS1), or peroxisome proliferator- activated receptor gamma (PPARγ)] than the CON diet ($p > 0.05$), except acetyl coenzyme A carboxylase 1 (ACC1), which was significantly reduced ($p < 0.05$; Figure 3(a)). However, feeding the largemouth bass on the LPHFC diet downregulated significantly DGAT1, ACC1, FAS1, and PPARγ than those fed on the CON diet ($p < 0.05$). Feeding the fish on the LPHF diet upregulated significantly the relative mRNA expression of genes related to lipid catabolism such as adipose triglyceride lipase (ATGL) and carnitine palmitoyltransferase (CPT) than CON diet (Figure 3(b); $p < 0.05$), but not hormone-sensitive lipase 1 (HSL1) ($p < 0.05$). Noticeable, feeding the largemouth bass on the LPHFC diet downregulated significantly the mRNA expression of ATGL, HSL1, and CPT genes ($p < 0.05$) to a similar level as in the CON diet ($p > 0.05$). Nevertheless, feeding the fish with the three experimental diets did not affect significantly the mRNA expression of peroxisomal acyl-coenzyme A oxidase 3 (ACOX3), peroxisome proliferator-activated receptor alpha (PPARα), and peroxisome proliferator-activated receptor beta (PPARβ) genes (Figure 3(b)).

3.4. Effects of Dietary L-Carnitine on Glucose and Protein Metabolite Contents. The largemouth bass fed on the LPHF and LPHFC diets decreased significantly the serum T-AA content than those fed on the CON diet (Figure 4(a); $p < 0.05$),

Table 2: The primers used in the for quantitative real-time PCR in the present study.

Gene	Sequences (5′ to 3′)	GenBank no.
DGAT1	F: CACGCCTCTTCTTGGAGAAC R: TGCCTGTGTTCAGCCAGTCAA	XM_038724648.1
ACC1	F: ATCCCTCTTTGCCACTGTTG R: GAGGTGATGTTGCTCGCATA	XM_038709737.1
FAS1	F: CAGCCCTTGACTCATTCCG R: CGCAGACTACGACCCGACAG	XM_038735140.1
PPARγ	F: CCTGTGAGGGCTGTAAGGGTTT R: TTGTTGCGGGACTTCTTGTGA	XM_038701594.1
ATGL	F: CCATGATGCTCCCCTACACT R: GGCAGATACACTTCGGGAAA	XM_038705351.1
HSL1	F: AGGACAGGACAGTGAAGAGTTGC R: CAGATAATTCTCATGGGATTTGG	XM_038725628.1
CPT	F: AGCCCCACCCCAACCTACCAG R: CGGCCCTCACGGAATAAACGC	XM_038705335.1
ACOX3	F: CTGGGCGATCTGCGTAATG R: TGGTGTTTTGCGTGGAGC	XM_038730772.1
PPARα	F: GCAGGAGCGTTGAAGGAC R: AGAACCCAGGGACGGACT	XM_038705497.1
PPARβ	F: AGCACCTCGCCATTTGTAATCT R: GGACCCCAATCTCCTTCGTC	XM_038734779.1
IL-1β	F: GAGAAATCAGCCACGGAGGAA R: TCATCCTGAACTTCCGTCATGT	XM_038733429.1
TGF-β	F: GCTCAAAGAGAGCGAGGATG R: TCCTCTACCATTCGCAATCC	XM_038693206.1
β-Actin	F: AAAGGGAAATCGTGCGTGAC R: AAGGAAGGCTGGAAGAGGG	KJ669299.1

DGAT1 = diacylglycerol O-acyltransferase 1, ACC1 = acetyl coenzyme A carboxylase 1, FAS1 = fatty acid synthase 1, PPARγ = peroxisome proliferator-activated receptor gamma, ATGL = adipose triglyceride lipase, HSL1 = hormone-sensitive lipase 1, CPT = carnitine palmitoyltransferase, ACOX3 = peroxisomal acyl-coenzyme A oxidase 3, PPARα = peroxisome proliferator-activated receptor alpha, PPARβ = peroxisome proliferator-activated receptor beta, IL-1β = interleukin 1 beta, TGF-β = transforming growth factor beta, and β-actin = beta actin.

with no significant differences in T-AA between them ($p > 0.05$). The fish fed on LPHF diet reduced significantly the whole-body protein content than those fed on the CON diet (Figure 4(b); $p < 0.05$). Interesting, feeding the largemouth bass on the LPHFC diet increased significantly the whole-body protein content ($p < 0.05$) to a similar level as in the CON diet ($p > 0.05$). The three experimental feeds used did not affect significantly the glycogen content in the liver of largemouth bass (Figure 4(c); $p > 0.05$). The fish fed on the LPHF diet increased significantly the serum glucose content compared to those fed on the CON diet (Figure 4(d); $p < 0.05$). However, feeding the largemouth bass on the LPHFC diet reduced significantly the serum glucose content than those fed on LPHF diet ($p < 0.05$) to a similar level as in the CON diet ($p > 0.05$). The fish fed on LPHF diet decreased significantly serum lactate content than those fed on the CON diet (Figure 4(e); $p < 0.05$). On the contrary, feeding the largemouth bass on LPHFC elevated significantly serum lactate content than those fed on the LPHF diet ($p < 0.05$) to a higher value more than those fed on the CON diet ($p < 0.05$).

3.5. Effects of Dietary L-Carnitine on Liver Health and Inflammation Response. The largemouth bass fed on LPHF diet increased significantly the ALT activity in the serum compared with those fed on the CON diet (Figure 5(a); $p < 0.05$), indicating a potential liver injury. However, feeding the fish on LPHFC diet decreased significantly the ALT activity in the serum than those fed on LPHF diet ($p < 0.05$) to a similar level as in the CON diet ($p > 0.05$). Feeding the largemouth bass with all the experimental diets did not affect significantly AST activity (Figure 5(b); $p > 0.05$).

The fish fed on LPHF diet upregulated significantly the expression of interleukin 1 beta (IL-1β) gene than those fed on CON diet (Figure 5(c); $p < 0.05$). However, feeding the largemouth bass on the HPLFC diet downregulated significantly the expression of IL-1β gene than those fed on LPHF diet ($p < 0.05$) to a similar level as in the CON diet ($p > 0.05$). Feeding the largemouth bass with the three experimental diets did not affect the expression of transforming growth factor beta (TGF-β) gene (Figure 5(c); $p > 0.05$).

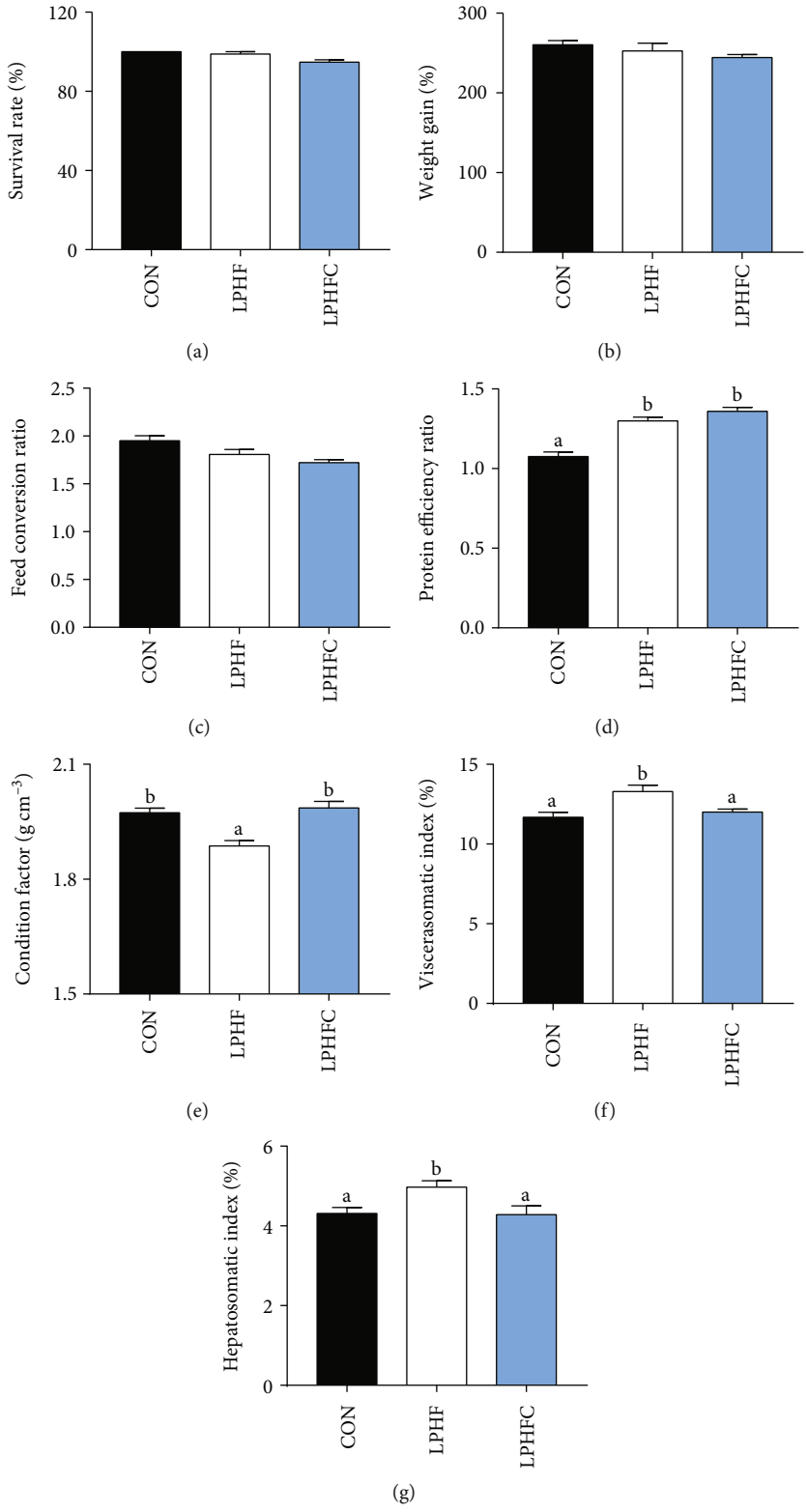


Figure 1: Effects of dietary L-carnitine on growth performance, feed utilization and organ indices in largemouth bass. (a) Survival rate; (b) weight gain; (c) feed conversion ratio; and (d) protein efficiency ratio. Results are represented as mean ± SEM ($n = 3$). (e) Condition factor; (f) viscerosomatic index; and (g) hepatosomatic index. Results are represented as mean ± SEM ($n = 6$). Mean values with different letters indicate statistical difference ($p < 0.05$).

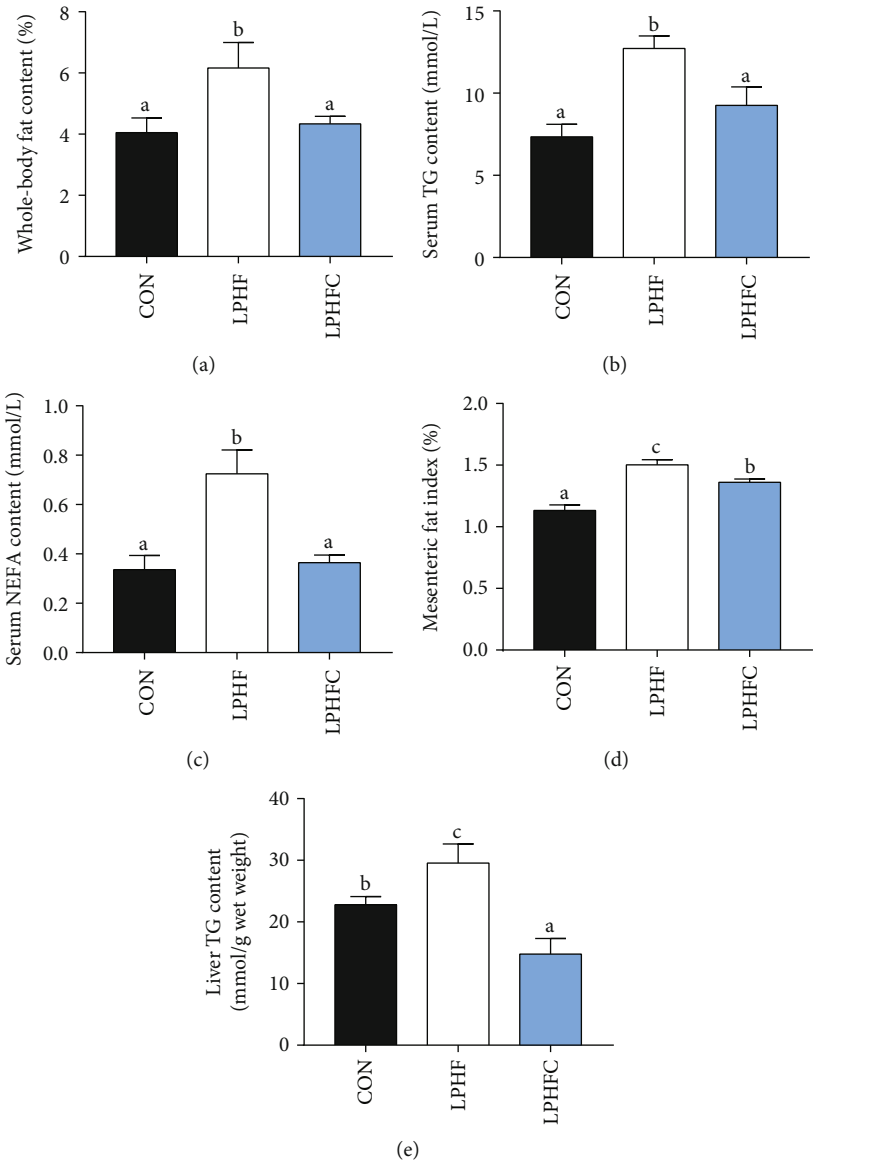
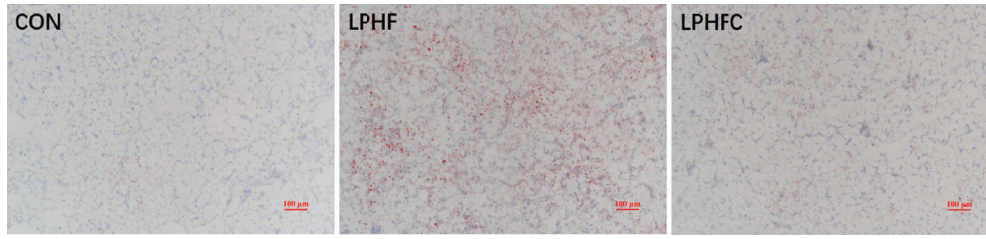


Figure 2: Continued.



(g)

Figure 2: Effects of dietary L-carnitine on lipid contents in largemouth bass. (a) Whole-body fat content; (b) serum triglyceride (TG) content; (c) serum non-esterified free fatty acids (NEFA) content; (d) mesenteric fat index; (e) liver TG content; (f) liver morphology; and (g) oil red staining of liver sections for fish fed on CON, LPHF, and LPHFC diets, respectively. Results are represented as mean ± SEM ($n = 6$). Mean values with different letters indicate statistical difference ($p < 0.05$).

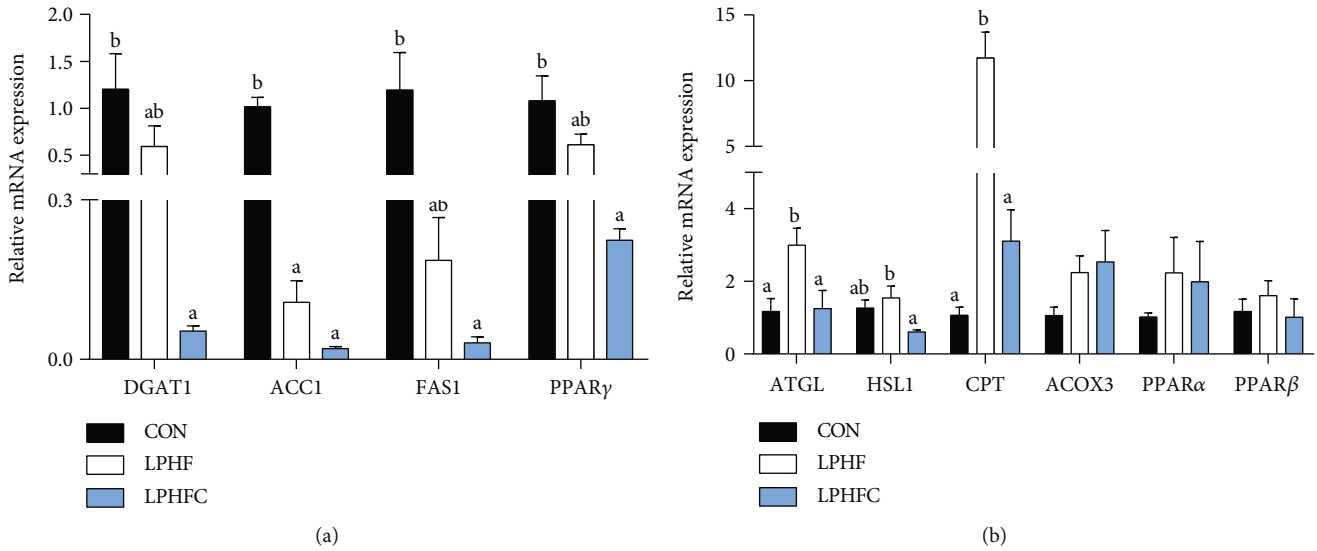


Figure 3: Effects of dietary L-carnitine on lipid metabolism-related gene expression in largemouth bass. (a) Lipogenesis-related genes (DGAT1 = diacylglycerol O-acyltransferase 1, ACC1 = acetyl coenzyme A carboxylase 1, FAS1 = fatty acid synthase 1, PPARγ = peroxisome proliferator- activated receptor gamma); (b) lipolysis-related genes (ATGL = adipose triglyceride lipase, HSL1 = hormone-sensitive lipase 1, CPT = carnitine palmitoyltransferase, ACOX3 = peroxisomal acyl-coenzyme A oxidase 3, PPARα = peroxisome proliferator-activated receptor alpha, and PPARβ = peroxisome proliferator-activated receptor beta). Results are represented as mean ± SEM ($n = 4$). Mean values for the same gene with different letters indicate statistical difference ($p < 0.05$).

3.6. Effects of Dietary L-Carnitine on Antioxidant Response. The largemouth bass fed on the LPHF diet reduced significantly the activities of SOD in the liver (Figure 6(a)) and serum (Figure 6(b)) than those fed on the CON diet ($p < 0.05$). However, the LPHF diet increased significantly the MDA content in the liver of largemouth bass than those fed on the CON diet (Figure 6(c)). Interesting, feeding the fish on the LPHFC diet increased significantly the SOD activities in the liver and serum and decreased significantly the MDA content in the liver than those fed on the LPHF diet ($p < 0.05$) to a similar level as in the CON diet ($p > 0.05$). Feeding the largemouth bass on the three experimental diets did not affect significantly the activities of CAT (Figure 6(d)) in the liver, T-AOC (Figure 6(e)) in the liver, and T-AOC (Figure 6(f)) in the serum ($p > 0.05$).

4. Discussion

In the present study, feeding the largemouth bass on the CON, LPHF, and LPHFC diets did not alter weight gain and FCR, although fish fed on LPHF and LPHFC increased PER compared to CON diet. Similarly, Chen et al. [38] reported an insignificant difference in specific growth rate of largemouth bass between fish fed on a 520 g kg^{-1} protein and 90 g kg^{-1} lipid and a 460 g kg^{-1} protein and 140 g kg^{-1} lipid diet. Likewise, Huang et al. [27] obtained similar FCR for largemouth bass fed on a 450 g kg^{-1} protein and 120 g kg^{-1} lipid and a 480 g kg^{-1} protein and 80 g kg^{-1} lipid. The reason for similar growth and feed utilization might be that the increased fat content while decreasing protein content of the diet caused the diets to provide similar energy to the fish fed on the CON diet. In fact, all the experimental diets used in the present study had a similar gross energy content. These results suggested that feeding the largemouth bass with CON, LPHF, and LPHFC diets did not affect the growth performance of the fish.

Our results showed that the largemouth bass fed on LPHF diet increased the fat content in the whole body, serum, and liver and enlarged liver size (HSI) and caused

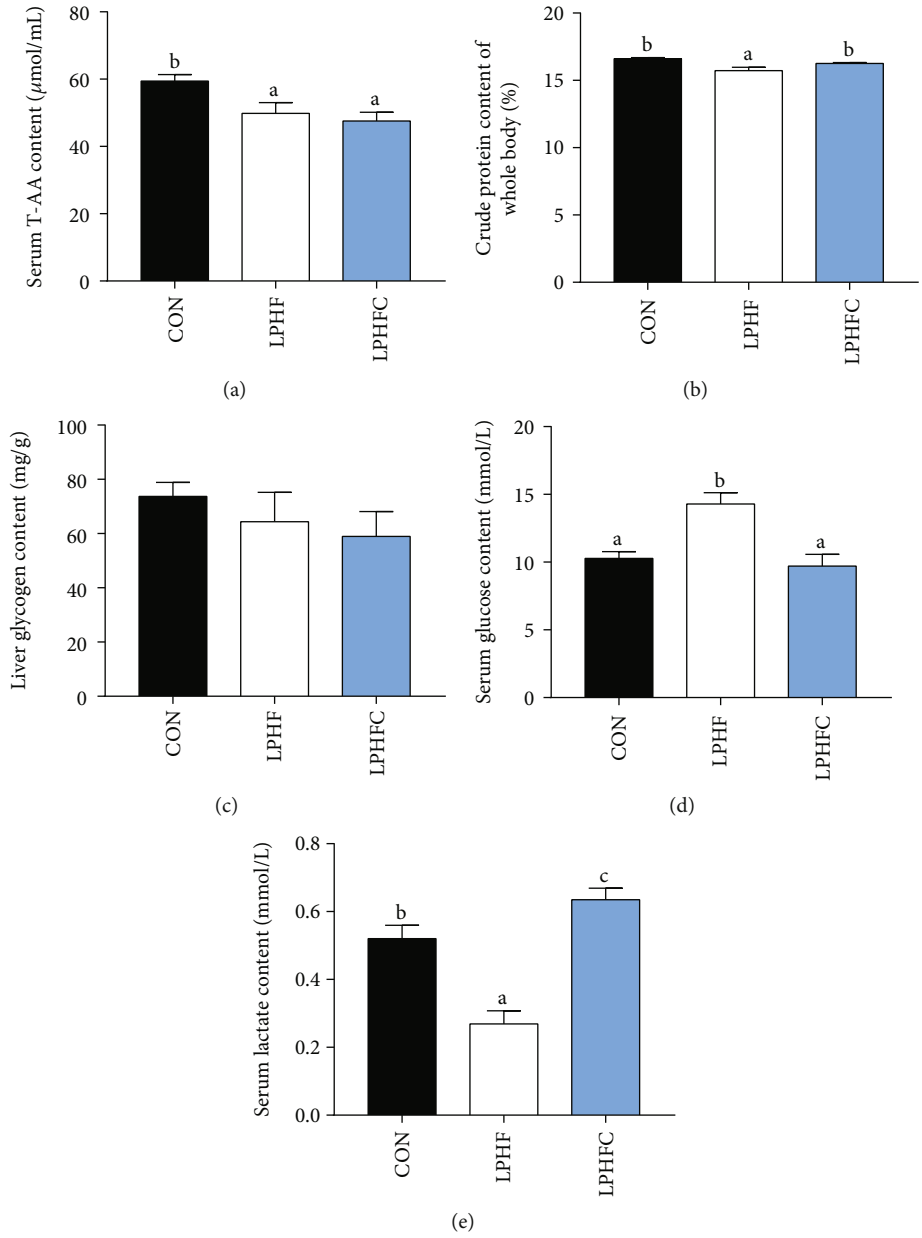


Figure 4: Effects of dietary L-carnitine on glucose and protein metabolites in largemouth bass. (a) Serum total amino acids (T-AA) content; (b) total protein content of the whole body; (c) liver glycogen content; (d) serum glucose content; (e) and serum lactate content. Results are represented as mean ± SEM ($n = 6$). Mean values with different letters indicate statistical difference ($p < 0.05$).

fat deposition (MFI). As expected, the L-carnitine supplementation reduced the fat deposition in the body and organs. Accordingly, the expression of genes related to lipogenesis such as DGAT1, ACC1, FAS1, and PPARγ were all downregulated by L-carnitine supplementation. The results on the ability of L-carnitine to reduce lipid in the body and organs are consistent with the previous findings obtained in a number of fish species such as channel catfish [39] and juvenile common carp (*Cyprinus carpio*) [40] and from some mammalian studies [41, 42]. The lipid-lowering effects of dietary L-carnitine supplementation are caused not only by increasing lipid catabolism, but also by inhibiting lipid synthesis at the transcriptional level as indicated in the present results. Moreover, our previous studies indicated that the effects of some lipid catabolic ligands such PPARα lipid reduction positively correlate with energy generating nutrients such as dietary lipid or protein content [43, 44]. Therefore, the effects of L-carnitine may also be related to dietary lipid content, such that the beneficial effects of L-carnitine might be greater in fish fed on HFD than those fed on diets with optimum required nutrients. Therefore, largemouth bass farmers feeding their fish with LPHF can use L-carnitine to reduce fat deposition in the body and organs for production of healthy fish.

It is well known that fish have a lower ability to use carbohydrates as an energy source [45] compared with

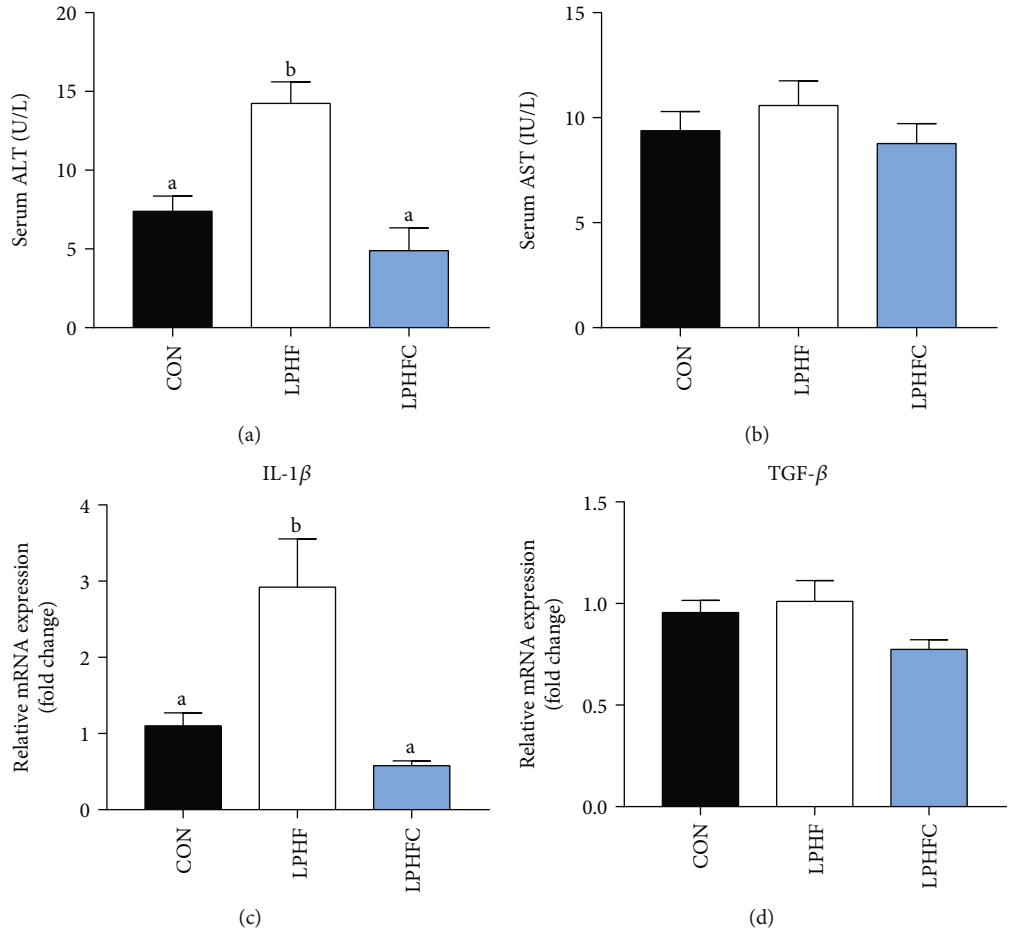


Figure 5: Effects of dietary L-carnitine on liver health and inflammation response in largemouth bass. (a) Serum alanine aminotransferase (ALT), (b) serum aspartate transaminase (AST), (c) mRNA expression of interleukin 1 beta (IL-1β), and (d) mRNA expression of transforming growth factor beta (TGF-β). Results are represented as mean ± SEM ($n = 6$). Mean values with different letters indicate statistical difference ($p < 0.05$).

mammals. However, fish have a higher ability to use protein as an energy source [46], causing high dietary protein requirement. One of the benefits of dietary L-carnitine in fish is to increase energy supply from lipid catabolism and save protein for energy production [47, 48]. Therefore, our present study aimed to assess whether the application of L-carnitine could strengthen the "protein sparing effect," especially in a diet with lower protein and higher lipid contents. The results in the present study showed that feeding the largemouth bass on LPHF diet increased body fat content and reduced protein deposition in body, suggesting that the high dietary fat content alone did not efficiently elevate protein deposition. Interestingly, the supplementation of L-carnitine in fish increased the whole-body protein content than those fed on LPHF diet. These results indicated that increasing fat content when done together with L-carnitine supplementation increased efficiently lipid catabolism and deposition of protein in the body, suggesting a "protein-sparing effect." Similarly, our previous study also indicated that dietary L-carnitine increased the whole-body protein content in zebrafish by upregulating the expression of mechanistic target of rapamycin (mTOR), which is the key signaling pathway in regulating protein synthesis [19]. Moreover, the protein-sparing effect of L-carnitine has been reported in Nile tilapia supplemented with 250 mg/kg L-carnitine in the diet, which increased protein content in the carcass [48]. Therefore, our present study and previous literature indicated that dietary L-carnitine promotes protein deposition in fish and saves protein as an energy source. Practically, our present study in largemouth bass helps to decrease feed cost by reducing protein content and increasing fat content in the diet during feed formulation. This feeding strategy may reduce economic costs in aquaculture taking into consideration that feed cost usually amounts to more than 50% of the operating costs depending on intensity of production.

It should be noted that our recent studies in Nile tilapia and zebrafish indicated that one side effect of increasing lipolysis is the reduction of carbohydrate catabolism, which normally causes glycogen deposition, based on energy homeostasis principles [21, 22]. However, in the present study, we did not observe any obvious glycogen accumulation in the liver of the fish fed on the LPHF or LPHFC diets. Moreover, the supplementation of L-carnitine even restored

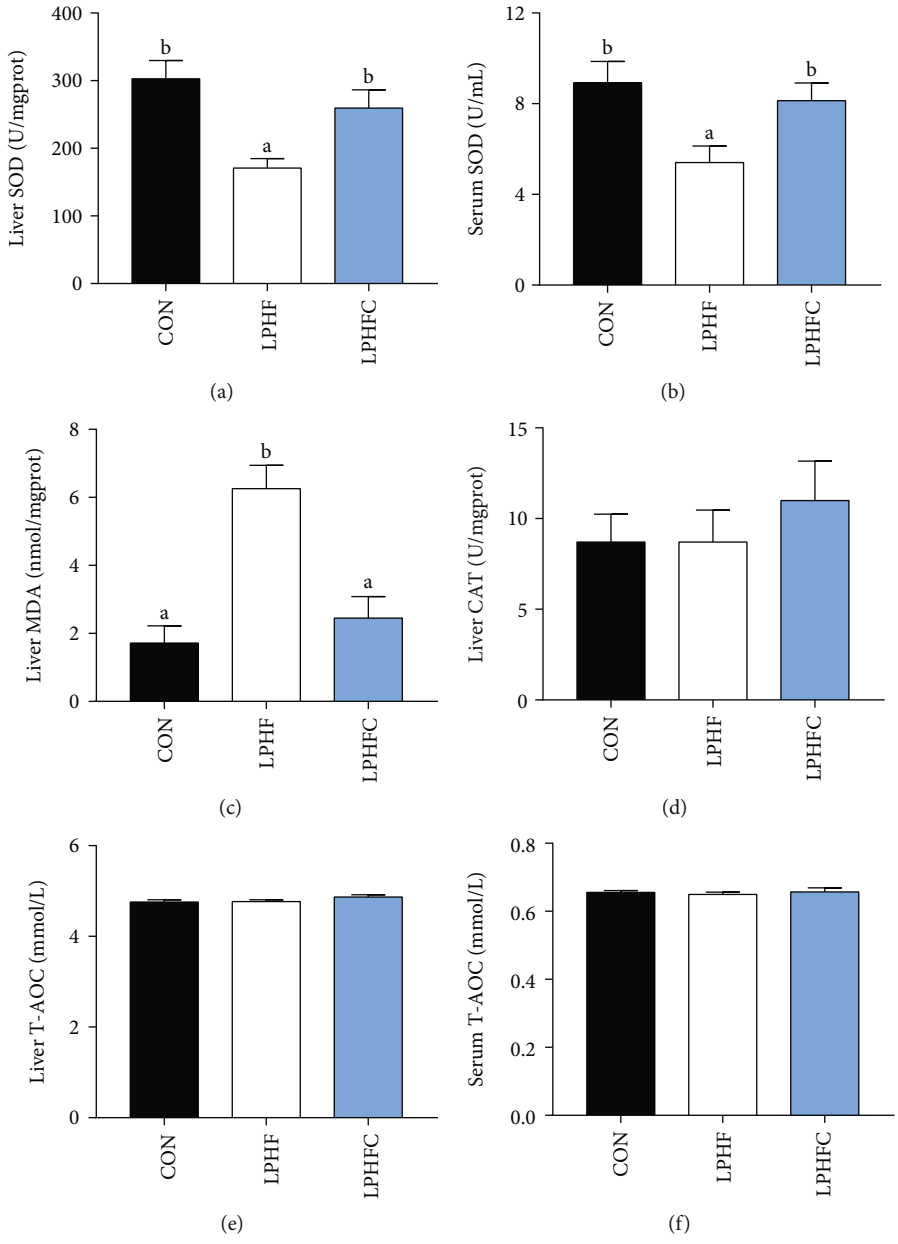


Figure 6: Effects of dietary L-carnitine on antioxidant response in largemouth bass. (a) Liver total superoxide dismutase (SOD); (b) liver MDA content; (c) serum SOD; (d) liver catalase (CAT); (e) liver total antioxidant capacity (T-AOC); and (f) serum T-AOC. Results are represented as mean ± SEM ($n = 6$). Mean values with different letters indicate statistical difference ($p < 0.05$).

the increase and reduction of serum glucose and lactate, respectively, caused by feeding fish on the LPHF diet, suggesting that the application of L-carnitine improved glycolysis in largemouth bass. Therefore, the mechanism for balancing nutrient homeostasis might be different among fish species, an aspect, which needs further investigation. In a nutshell, L-carnitine supplementation in low protein and high fat content diet strengthens the "protein sparing effect" without affecting carbohydrate catabolism in juvenile largemouth bass.

The literature provided enormous evidences that feeding fish with HFD for long-term not only induce excess fat deposition, but also trigger impaired immune and antioxidant responses [49–51]. Accordingly, some studies reported that increased lipid catabolism by dietary L-carnitine supplementation [34] or PPARα activators addition [52], elevated antioxidant and immune ability in fish, while the inhibition of L-carnitine synthesis reduced resistance to bacteria pathogen and environmental stress [53]. Similar results on the protective effect of L-carnitine on immunity and toxicity were also reported in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) [54] and tilapia hybrids (*Oreochromis niloticus* x *O. aureus*) [55]. In the present study, we found that the LPHF diet caused potential liver damage in largemouth bass as evidenced by the increased ALT activity in the serum, higher expression of inflammatory factor (IL-

1β) gene, and lower hepatic SOD activity compared with fish fed on the CON diet. However, the L-carnitine supplementation alleviated the adverse effects in largemouth bass by reducing ALT activity and the expression of IL-1β gene and increased SOD activity, similar to fish fed on the CON diet. The ability of L-carnitine to alleviate liver damage, inflammation, and antioxidant responses is related to its capacity to increase lipid catabolism. Elevation of lipid catabolism enhances immune response because it supplies energy required during immune response [34, 52]. Moreover, feeding fish with HFD induce oxidative stress attributed to lipotoxicity, which is caused by overload of lipid [56]. Therefore, the increased antioxidant ability after L-carnitine supplementation is related to its efficient roles in elevating lipid catabolism and reducing lipid overload. Our results indicate that the adverse effects of reducing protein and increasing lipid content in fish diets on antioxidant and immunity responses can be alleviated by L-carnitine through decreasing lipogenesis and increasing lipid catabolism.

5. Conclusion

In summary, our results indicated that reducing protein and increasing fat contents in largemouth bass diet cause fat accumulation and elicit adverse effects by impairing antioxidant and immunity responses and consequently damage liver health. L-carnitine supplementation in such diets alleviates the adverse effects and improves deposition of protein in the whole body. Therefore, we recommend to supplement the low protein and high lipid contents diets in largemouth bass with L-carnitine during feed formulation.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Acknowledgments

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